THE KANSAS UNIVERSITY SCIENCE BULLETIN.

Vol. IX, No. 20—September, 1915.

(Whole Series, Vol. XIX, No. 20.)

CONTENTS:

DIFFERENTIATION OF THE OAKS BY HISTOLOGICAL METHODS, *F. W. Mulsow.*

PUBLISHED BY THE UNIVERSITY,

LAWRENCE, KAN.

Entered at the post-office in Lawrence as second-class matter.

52496

KANSAS STATE PRINTING PLANT.
W. R. SMITH, State Printer.
TOPEKA. 1915.

THE KANSAS UNIVERSITY SCIENCE BULLETIN.

VOL. IX, No. 20.] SEPTEMBER, 1915. [WHOLE SERIES VOL. XIX, No. 20

Differentiation of the Oaks by Histological Methods.

F. W. MULSOW.

Plates LX-LXIV.

IN this work four species of native oaks are compared as to their histological structures and characteristics. The species studied are *Q. rubra, Q. schneckii, Q. coccinea* and *Q. macrocarpa,* as classified by Britton and Brown. Branches, leaves and acorns from one tree only of each species were studied throughout their essential parts. But in the study of *Q. rubra* and *Q. schneckii* three different trees were studied in a few of their structures peculiar to themselves, in order to find out the constancy of their characteristic structures. Parts of the leaves were embedded in paraffin and sectioned with the rotary microtome; also, some of the leaves were bleached for studying the cell structure and leaf venation. Parts of the branches were placed in dilute glycerine, this making them easier to section. The double stain of safranin and hæmatoxylin was used in differentiating the various tissues. The staining for temporary mounts may be carried out in one operation by mixing equal parts of each stain and placing the sections in this mixture for a few minutes. After washing in seventy per cent alcohol they are ready for studying in dilute glycerine. For best results the stain should be made up fresh just before using.

Received for publication June 10, 1915.

THE LEAF.

The gross structure of the leaves is quite different in each of these oaks. The leaf of *Q. rubra* is somewhat smaller than either of the others, has a smooth upper surface and a dark green color. It is not so deeply lobed as the others and is less flexible. The leaf of *Q. schneckii* is of a light green color and quite deeply lobed, and the lobes are more divided into smaller lobes than any of the others. It is much thinner and hence more flexible than any of the others. The leaf of *Q. coccinea* is of a dark green color with a glossy upper surface. It is longer than the others and has fewer lobes, which are nearer at right angles to the midrib than in any of the others. *Q. macrocarpa* has a leaf quite different from any of the others. It is of a light green color and has many hairs on the lower side. The young leaves of all these oaks have hairs on both the upper and lower surfaces. The comparative sizes and shapes are shown in figures 1, 2, 3 and 4.

The Bleached Leaf.

One of the first differences noticed between the leaves of the different oaks was the time required to bleach the leaves. The leaf of *Q. macrocarpa* was cleared in the chloral hydrate in about two days, and took about the same time to clear in the potassium hydrate. The leaf of *Q. schneckii* and *Q. coccinea* was cleared in the chloral hydrate in about five days and in the potassium hydrate in two days. The leaf of *Q. rubra* was cleared in the chloral hydrate in seven days and in the potassium hydrate in three days, thus taking about twice as long to bleach as it took to bleach the leaf of *Q. macrocarpa.* The venation of the leaves was examined in the bleached leaves, but no constant difference could be noticed.

Upper Epidermis.

There is a noticeable difference in the size of the upper epidermal cells. There being 2500 cells per square millimeter in the leaf of *Q. macrocarpa,* 1900 in the leaf of *Q. schneckii* and *Q. coccinea,* and about 1650 cells per square millimeter in *Q. rubra;* the greatest difference being between *Q. macrocarpa* and the others, thus easily distinguishing *Q. macrocarpa* from the others. (See figs. 5, 6, 7 and 8.) A cross section of the leaf shows the upper epidermis of *Q. ruba* to be the thickest, being about .03 mm. thick. The upper epidermis is about .028

mm. thick in *Q. coccinea*, .02 mm. in *Q. schneckii*, and about .016 mm. in *Q. macrocarpa*. The cell walls of *Q. rubra* are also slightly thicker than in any of the others. (For the comparison, see figs. 13, 14, 15, 16.)

Leaf Parenchyma.

The palisade cells of *Q. macrocarpa* are slightly more numerous than in any of the others, there being 22,000 per sq. mm. in *Q. macrocarpa*, 21,100 per sq. mm. in *Q. rubra*, 20,200 per sq. mm. in *Q. schneckii*, and 16,600 per sq. mm. in *Q. coccinea*. (See figs. 5, 6, 7, 8.) The cross section of the leaf shows the palisade cells of *Q. schneckii* to be shorter than the others, being .035 mm. long, while in the other leaves they are about .045 mm. in length. This seems to account for the thinness of the leaf of *Q. schneckii*. (See figs. 13, 14, 15 and 16.)

Lower Epidermis.

The sizes of the cells in the lower epidermis are nearly the same in all except in *Q. macrocarpa*, there being 10,960 cells per sq. mm. in *Q. macrocarpa*, and in the others they run thus from 4920 in *Q. schneckii* to 4900 in *Q. rubra* and 4870 per sq. mm. in *Q. coccinea*. The thicknesses of the lower epidermis are various in the different species. In this respect any measurements give *Q. rubra* .016 mm., *Q. schneckii* .015 mm., *Q. coccinea* .012 mm., and *Q. macrocarpa* .010 mm. The lower epidermis of *Q. macrocarpa* also has persistent hairs in tufts, while in the others the hairs fall off when the leaves are quite young.

Stomata.

The stomata are found on the lower side of the leaf. They vary among the different species as to number, size and shape. The number of stomata per square millimeter was found by taking the average of twenty-five fields from various parts of the leaf, except where a vein occupied a whole field such a field was not counted. The fields counted were .09 sq. mm. in area. The number of stomata per square millimeter was found to be 375 in *Q. schneckii*, 500 in *Q. coccinea*, 515 in *Q. rubra*, and 819 in *Q. macrocarpa*. The stomata of *Q. schneckii* are of two sizes, the larger size being the more numerous and of elliptical shape, while the smaller size are almost round. The stomata of *Q. rubra* and *Q. coccinea* are almost round, but in *Q. macrocarpa* the stomata are quite elliptical and smaller

than in any of the others. The length of the opening is nearly one-half the length of the guard cells in *Q. schneckii,* about two-fifths the length of the guard cells in *Q. rubra* and *Q. coccinea* and one-third the length of the guard cells in *Q. macrocarpa.* The length of the larger stomata of *Q. schneckii* is about .03 mm., and the length of the smaller sized stomata of *Q. schneckii* is about the same as the length of the stomata of *Q. rubra* and *Q. coccinea,* which is about .024 mm. In *Q. macrocarpa* the stomata are about .023 mm. long but are much narrower than in the others, hence they look much smaller. The proportionate lengths and shapes are shown in figures 9, 10, 11, 12.

Leaf Cross-section.

In the cross-section of the leaves it is noticed that the leaf of *Q. rubra* is slightly thicker than the others, it being .16 mm. thick, while *Q. coccinea* is about .15 mm. thick and *Q. schneckii* and *Q. macrocarpa* are about .13 mm. thick. The thinness of the leaf of *Q. schneckii* seems to be due to the thinness of the palisade parenchyma, and the thinness of the leaf of *Q. macrocarpa* seems to be due to the thinness of the spongy parenchyma. The spongy parenchyma, as well as the palisade parenchyma, is more compact in *Q. macrocarpa* than in the others. (See figs. 13 to 16, inclusive.)

Midrib.

In the cross-section of the midrib there are three layers of phloëm tissue, a fact characteristic of the oaks (Solereder). There is also xylem tissue with the middle layer of phloëm. The xylem is above the phloëm in the middle layer, or the phloëm is said to face the upper surface of the leaf. In tracing this middle layer of xylem and phloëm from the base of the leaf to the apex it is found that the xylem disappears, or unites with the upper layer of xylem before the phloëm does. The middle layer of phloëm always unites with the upper layer of phloëm, but in different ways in the different species, and also at different distances from the base of the leaf.

In *Q. rubra* this layer of phloëm usually extends about half the distance from the base to the apex. At this point the last or uppermost large vein usually branches off from the midrib. The upper layer seems to divide in the middle and the ends bend down. At the same time the middle layer of phloëm

divides longitudinally, and the inner ends bend up to meet the ends of the upper layer which are bent down. This middle layer of phloëm also extends out into a few of the larger side veins, but only to a distance of about 4 mm. This method and place of ending was the same in the three trees of *Q. rubra* studied. (Figs. 17-19.)

In *Q. schneckii* this middle layer of phloëm extends about two-thirds the distance from the base to the apex of the leaf, which is usually the point where the vein of the upper largest lobe separates from the midrib. The middle of the phloëm ends by forming the partition between the midrib and the side vein branching from it. This usually unites with the upper layer just before the partition is formed between the vein and the midrib. The upper layer of phloëm bends down in the middle to unite with this middle layer of phloëm, which is almost divided longitudinally to unite with the two portions of the lower phloëm layer which form the lower layers of the midrib and vein. This middle layer of phloëm also extends out into the side veins about one-half the distance to the apex of the lobe. These characteristics were the same in the three trees of *Q. schneckii* studied. (See figs. 23-25.)

In *Q. coccinea* this middle layer does not end with the larger side veins as was the case with the other leaves studied. It extends about three-fourths the distance to the apex of the leaf. It first divides longitudinally, then each part unites with the outer ends of the upper layer. This middle layer extends up the side veins about two-thirds of their length. (See figs. 20, 21, 22.)

In *Q. macrocarpa* this layer of phloëm extends nearly three-fourths the distance to the apex of the leaf, where small veins branch off. It forms the partition between the veins and the midrib, and there are usually two veins branching off at the same place or nearly so. (See figs. 26 to 30.) This phloëm extends into only the two large side veins, and extends up them about two-thirds the distance to the apex of the lobes.

STEM.

In cross sections of the current year's growth of the stem the division into vascular bundles is more noticeable in *Q. ruba* and *Q. coccinea* than in *Q. schneckii* and *Q. macrocarpa*. In *Q. macrocarpa* the cork-cambium usually produces enough cork

in the first year's growth to break up the epidermis, while in the others the epidermis remains unbroken for at least five years, and usually much longer. The number of water tubes per unit area is much less in *Q. macrocarpa* than in the others. There is the largest number of water tubes in *Q. schneckii.* It also has a smaller proportion of pith and parenchyma of the cortex than the others. The bast and phloëm in *Q. schneckii* and *macrocarpa* are almost entire, while in the others the bast and phloëm are divided. (See figs. 31 to 34.)

Cross sections of the older stems show that the water tubes in *Q. schneckii* occupy a much larger space and are more scattered throughout each year of growth than in any of the others. In *Q. rubra,* and especially *Q. coccinea,* the water tubes are usually found in the first part of each year's growth, and much less scattered through the whole year's growth than in *Q. schneckii.* In *Q. macrocarpa* there are not so many water tubes and they are not as large or disperse as in the others. (Figs. 43 to 46.) In tangential sections there is a difference in the number of medulary rays per unit area. Thus in *Q. macrocarpa,* which has the largest number per sq. cm., there are about 108 rays per sq. cm. The rays are also shorter and broader than in the others. In *Q. rubra* there are 96 rays per sq. cm. A large per cent of these are longer than in *Q. macrocarpa,* but the per cent of long rays is not as large as in *Q. coccinea,* which has the least number of rays per sq. cm., there being about 57 per sq. cm. The rays in *Q. coccinea* are also more slender. In *Q. schneckii* there are 75 rays per sq. cm., and no very long rays. (Figs. 47 to 50.)

Macerations of the wood and bast fibers were made by gently heating sections of each in Schultze's macerating fluid, in order to study the comparative lengths of the fibers. The wood fibers of *Q. macrocarpa* are on an average shorter than the others, all of which are about the same length; however, those of *Q. schneckii* are slightly broader than the others. The bast fibers are on an average about the same length, except in *Q. coccinea,* where they are about twice as long as in the others. Thus in *Q. coccinea* the bast fibers are as long as 1.4 mm.; in the others they average about .6 mm. in length. (Figs. 35 to 42.) There was no noticeable difference in size or character of the cells of the wood parenchyma of these four oaks.

ACORNS.

The size and structure of the acorns is noticeably different in all of these oaks; yet there is a somewhat close resemblance between the acorns of *Q. rubra* and *Q. schneckii.* The cup in *Q. schneckii* is deeper and has coarser bracts; the acorn is not so large, and is more pointed. The acorn of *Q. macrocarpa* is much larger than the others, and the acorn of *Q. coccinea* is much smaller. The shell of the acorn of *Q. rubra* is thicker than the others. The inner surface is covered with hair-like outgrowth in all except in *Q. macrocarpa,* which, however, has a thin layer of cells forming a lining membrane. The shell wall of *Q. coccinea* is much thinner than the others. (Figs. 55 to 58, inclusive.)

The starch grains were larger in *Q. rubra* and *Q. schneckii,* being about .0076 mm. in diameter, and in the others about .0062 mm. The acorns are more numerous on the branches of *Q. coccinea* and *Q. rubra.* For relative sizes and shapes see figs. 51 to 54.

CONCLUSIONS.

From this study of the oak it appears that there are enough differences in the various tissues of the oaks to enable one to distinguish the species by histological methods. There were found differences in the leaf sufficient to distinguish the different species, and in addition to this there are differences in the stem and acorns which would enable one to further distinguish the species.

BOTANICAL LABORATORY.
UNIVERSITY OF KANSAS.